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(71) Applicant: TEMPLE UNIVERSITY [US/US]; 406 University Service Building, Philadelphia, PA 19122 (US).

(72) Inventor: JAMESON, Bradford, A. ; 810 W. Sedgwick Street, Philadelphia, PA 19119 (US).

(74) Agents: HOSCHEIT, Dale, H. et al.; 1001 G Street, N.W., Eleventh Floor, Washington, DC 20001-4597 (US).

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(57) Abstract

A synthetic peptide which is conformationally restricted by means of intramolecular bonding can bind to proteinaceous binding partners. Such binding is not seen in the absence of conformational restriction. The peptides of the invention mimic the biological activities of the proteins of the immunoglobulin superfamily of proteins, providing therapeutic and diagnostic molecules for a broad range of diseases. Methods for molecular modeling to create such peptides employ known structures of other superfamily members.

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PEPTIDE ANALOGS TO PROTEINS OF THE IMMUNOGLOBULIN SUPERFAMILY

BACKGROUND OF THE INVENTION

The CD4 protein, a member of the immunoglobulin superfamily of proteins, has historically served as a phenotypic marker for the separation of helper/inducer T cells from mixed lymphocyte populations. In the context of the helper/inducer T cells, it plays an essential role in the recognition of foreign antigen by helper T cells. For a foreign antigen to stimulate helper T cells, it must be processed by an antigen-presenting cell and presented on the surface of these cells in the context of the polymorphic class II major histocompatibility complex (MHC). It has recently been shown that the CD4 protein binds directly to the class II MHC (Doyle et al., 1987).

In addition to its role in antigen recognition, the CD4 protein has been shown to directly interact with the gp120 envelope protein of HIV (McDougal et al., 1986). Furthermore, it has been demonstrated that the cell surface expression of the CD4 protein on a non-permissive human cell line (HeLa), can enable the virus to bind and infect this cell type (Maddon et al., 1986). Monoclonal antibodies to the CD4 protein can prevent HIV from infecting permissive cells (Klatzman et al., 1984; Dalgleish et al., 1984; Sattentau et al., 1986). Finally, soluble constructs of the CD4 protein show a concentration-dependent inhibition of HIV infection (Lasky et al., 1987). Taken together, this evidence shows that the CD4 protein can serve as a receptor for HIV binding. Remarkably, the subset of CD4-derived monoclonal antibodies that most potently inhibits HIV binding also inhibits CD4-dependent antigen stimulation of helper T cells (Biddison et al., 1982; 1983; 1984). This observation suggests that th gp120 binding site on the

CD4 protein may also serve as a site of interaction for the class II MHC.

Although the gp120 protein binding surface on the CD4 protein has not yet been directly identified, it is known that expression constructs of the V_1 domain of the CD4, containing the first 104 residues, are able to bind directly to the gp120 protein with high affinity (Arthos et al., 1989). Furthermore, a series of genetic mutation analysis studies, using either mouse/human amino acid replacements, Ser-Arg insertions or saturation mutagenesis, indicates that mutations that cluster within CD4 residues 38-55 abrogate the binding of the gp120 protein (Peterson and Seed, 1988; Clayton et al., 1988; Mizukami et al., 1988; Arthos et al., 1989). These data suggest that this region (38-55) is involved in the direct contact of the gp120 protein.

The V_1 domain of CD4 bears strong homology to the light chain variable region of an antibody (Littman, 1987) and biological data indicates that the actual folding architecture of this domain resembles that of an Ig protein (Jameson et al., 1988; Peterson and Seed, 1988). The antibody light chain variable domain contains three (hypervariable) substructures that are directly involved in contacting an antigen, referred to as complimentarity determining regions (CDR1, CDR2 and CDR3). The CD4 protein region 38-55, implicated in binding to gp120, falls within a region analogous to the CDR2 of an antibody. If generic structure/function relationships exist among members of the Ig-superfamily of proteins, then this is not a surprising observation. On the other hand, it is then odd that mutations in the CDR1 and CDR3 domains did not affect the gp120 interaction with CD4. In this context, it should be noted that carboxy terminal deletions of the V_1 construct that extend into the CDR3 domain, i.e. extend beyond residue 101, result in a biologically inactive protein product (Arthos et al., 1989). Although many different explanations for this result exist, it is interesting that residue 101 defines the end of the CDR3 domain. It has also been reported that a derivatized synthetic peptide derived from the CDR3 region, residues 81-92, is able to inhibit virus-induced syncytium formation, virus binding and

virus infection (Lifson et al., 1988; Eiden 1989). Because the parent peptide (with intact side chains) does not show any biological activity, this result is difficult to interpret. Hayashi, et al. (1989), however, report that a larger (non-derivatized) peptide spanning the CDR3 domain, residues 68-130, was an effective inhibitor of virus infection and virus-induced syncytium formation. They also found that a smaller peptide, residues 84-92, although less active, inhibited virus-induced cell fusion and infection.

In a series of recent studies, evidence has been presented that there is considerable overlap between the gp120 binding site on the CD4 protein and the binding site for the MHC class II, also a member of the Ig-superfamily of proteins (Lamarre et al., 1989; Clayton et al., 1989). Lamarre et al. (1989), using genetic constructs, replaced the human CD4 residues 38-42 with the equivalent residues from the mouse L3T4 and found that only gp120 binding was abrogated, but not that of MHC class II. This observation suggests that a small portion of the binding surface of the CD4 protein may be uniquely utilized in the interaction with the gp120 protein.

Because of the crucial roles of CD4 in the processes of HIV-1 infection and stimulation of helper T-cells by foreign antigens there is a continuing need in the art for structural information about CD4 which can be used to manipulate these processes. There is a need in the art for substances which can specifically inhibit these biological processes to ameliorate diseases such as AIDS and autoimmune diseases such as lupus, multiple sclerosis, and arthritis.

SUMMARY OF THE INVENTION

It is an object of the invention to provide a synthetic peptide analog to a conformational domain of a protein which is a member of the immunoglobulin superfamily of proteins.

It is another object of the invention to provide a method of designing a peptide analog to a conformational domain of a member of the immunglobulin superfamily of proteins.

It is still another object of the invention to provide a method of producing a peptide analog to a conformational domain of a member of the immunoglobulin superfamily.

It is yet another object of the invention to provide methods of treating human diseases employing the peptide analogs of the invention.

It is an object of the invention to provide methods and kits for use in the diagnosis of human diseases, said methods and kits employing the peptide analogs of the invention.

It is also an object of the invention to provide antibodies specific for the peptide analogs of the invention, said antibodies useful in the treatment and diagnosis of human diseases.

These and other objects of the invention are provided by one or more of the embodiments described below. In one embodiment a synthetic peptide is provided which comprises an amino acid sequence corresponding to a portion of a member of a family of proteins known as the immunoglobulin superfamily, said member having the ability to bind specifically to a second protein, said peptide having a restricted conformation and having the ability to bind to the second protein, said peptide being free of benzylated and methylbenzylated amino acids.

In another embodiment of the invention a method is provided of designing a peptide analog to a conformational domain of a member of a family of proteins known as the immunoglobulin superfamily of proteins, comprising:

providing a first model of a conformational domain of a first member of the immunoglobulin superfamily of proteins;

generating by homology modeling a second model of an homologous domain of a second member of the superfamily to form a second model of a molecule having a primary structure corresponding to a portion of the second member;

determining a distance between two parts of th molecule in the second model at conformational equilibrium;

modifying the primary structur of the molecule to restrict the distance between said two parts of the molecule to the determined distance.

In yet another mbodiment a method is provided of producing a peptide having a restricted conformation, comprising:

providing a peptide comprising a portion of a member of a family of proteins known as the immunoglobulin superfamily of proteins;

determining conformational equilibrium of the portion of the protein in the whole protein;

introducing a covalent modification into the peptide to restrict a distance between two parts of the peptide to a distance between the two parts of the peptide at conformational equilibrium.

Other embodiments will be clear to those of skill in the art from the disclosure which follows. The present invention provides the art with the ability to design conformational analogs to proteins of the immunoglobulin superfamily without first having to obtain a crystallographic structure for each protein. The conformational analogs are better able to mimic the biological properties of the proteins than are linear peptides. In addition, the synthetic analogs are more conveniently prepared than larger protein molecules having similar biological activity because the larger molecules must be prepared from recombinant organisms or natural sources and subsequently purified.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 schematically shows the V_1 domain of the CD4 protein and its component subdomains. Also shown are synthetic peptide analogs of the CDR2 and CDR3 subdomains and their biological activities.

Figure 2 shows the inhibition of HIV-1 binding to $CD4^+$ human cells by human and mouse CDR3 analogs. The effect on this inhibition of an unfolding and refolding procedure performed on the analogs is also shown.

Figure 3 shows a model of the folded structure of CD4 based on the known crystallographic structure of the light chain variable domain of a human Fab homodimer immunoglobulin fragment.

Figure 4 shows chemical reactions which can be used to internally cross-link a peptide of the invention via an amide bond between a lysine and a glutamic acid residue.

DETAILED DESCRIPTION

The present invention employs a "rational design" approach to the synthesis of conformational analogs of CDR-like domains of the Ig-superfamily of proteins. Traditional syntheses of the linear amino acid sequence of these structures results in products that are either biologically inactive or, at best, marginally active. The approach disclosed here employs the creation of a design template, through molecular modeling. Using both distance and geometric constraints imparted through measurements of the subdomains within the calculated structure, constraints are artificially introduced, e.g. disulfide bonds, to limit the conformational freedom of a synthetic peptide that incorporates the relevant amino acids.

The present invention is based on the observation that the CDR-like domains within the V_1 domain of the CD4 protein fold together to form a continuous binding surface, as is observed in an antibody. These results suggest that members of the Ig-superfamily utilize these CDR-like subdomains to create generic binding surfaces specific to each member of the family. With respect to the CD4 protein, we have created a molecular model of the three-dimensional structure of the V_1 domain based on the general backbone folding patterns of the superfamily. The techniques we have utilized in generating the CD4 structure can be generally applied to all members of the superfamily. The CD4 structure created in this manner has been successfully used as a template for designing conformationally-restricted synthetic analogs of the CDR-like domains. One particular conformationally-restricted synthetic peptide analog is a CDR3 analog of the CD4 protein (residues 84-101) capable of potently inhibiting HIV from binding to CD4-positive cells.[1] Another peptide analog comprises residues 42-54 of th CDR2 domain of CD4 protein. The techniques disclosed here are applicable to the universal design and synthesis of CDR-type analogs for all members of the Ig superfamily.

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1 The numbering of th amino acids of CD4 protein is according to Hussey, R.E., Nature, vol. 331, pp. 78-81 (1988).

According to the present invention domains of members of the immunoglobulin superfamily are modeled using a known structure from another member of the family. While the applicant does not wish to be bound by any particular theory, it is believed that the CDR-like domains of the superfamily members form a generic binding surface for interaction with other proteins. The compositions and functional properties of the members of the immunoglobulin (Ig) superfamily are extraordinarily diverse, ranging from neural adhesion to presentation and recognition of foreign antigens by the immune system. However, the backbone folding patterns of the proteins of the Ig superfamily are highly conserved. The techniques disclosed herein can be applied to any member of the Ig superfamily using the known structure of any Ig superfamily member as a starting point.

A first model of a conformational domain of a first superfamily member is generally obtained from nuclear magnetic resonance or high resolution X-ray crystallographic analyses. See, e.g., Epp, et al., supra. The side chains of the first superfamily member are stripped and replaced with those of the domain of the second family member to be determined. Alternatively, this can be described as superimposing the amino acid sequence of the second member on the backbone of the first member. Homology modeling methods are generally known by those of ordinary skill in the art. See, Jameson (1989), Nature, vol. 341, pp. 465-466. This modeling is most conveniently accomplished using computers and software which are commercially available. The backbone of the model is then adjusted after the side chains are oriented by a successive series of energy minimization calculations to form a second model. The adjustments include both mechanical and dynamic adjustments which are completed when the entire domain reaches conformational equilibrium. Wh n the adjustments are made, the second model can be used to design peptide analogs.

The analogs of the invention generally have the same amino acid sequence as the second member which is being modeled. However, a covalent modification is introduced to restrict th analog to the conformation (or one close to it) displayed in the model. Gener-

non-contiguous parts of the amino acid chain according to the second model. Then a chemical moiety is introduced to fix that determined distance in the analog. For example, a 5 to 6A distance can be fixed using a disulfide bond. Cysteine residues can be introduced at the appropriate positions in the model and then the new model is tested for its ability to mimic the structure observed in the mode of the second family member.

The use of artificially introduced cysteine residues to create a disulfide bridge is one way to conformationally restrict the peptides. Disulfide bonds, however, are intrinsically unstable and it is impossible to ever get a homogeneous solution of intradisulfide-bonded species without concomitant mixed disulfides. The disulfide bridges can be replaced in biologically active peptides by stable covalent bonds. There are several strategies that can be utilized in the covalent closure of the peptides, two of these strategies are described below.

The peptide can be internally crosslinked via the side chains of a lysine (epsilon amino group) and the carboxylic acid function of a glutamic or aspartic acid side chain, thus creating an amide bond. The peptide is synthesized according to standard procedures on a low substitution (0.2 mM/gm or less) para-methylbenzhydrylamine resin. The first residue added to the resin is an N-alpha-tBOC, epsilon-fMOC lysine. The rest of the peptide is continued normally using tBOC chemistry until the final residue is added. The last residue to be added is a Z-protected glutamic acid, where the carboxylic acid moeity is protected with a tert-butyl group. Treatment of the peptide-resin with piperdine/DMF removes the fMOC group from the epsilon amino group of the initial lysine without affecting any other protection groups and subsequent treatment with TFA removes the protection of the carboxylic acid group of the glutamic acid. Following neutralization, the peptide is covalently closed using a standard diimide mediated coupling reaction. It should be emphasized that this is only one of th ways in which the synthetic peptide can be covalently closed.

Oth r fMOC/tBOC strategies include covalent closure of the peptide between two free amino groups utilizing toluene-2,4-diisocyanate (TDI), a heterobifunctional cross-linker. The

methyl group of the aromatic ring of TDI prevents the isocyanate group in the 2 position from reacting at a pH 7.5 or below, whereas the isocyanate group in the para position is highly reactive. A shift in pH to greater than 9.0 will initiate a reaction with the isocynate group in the 2 position, thus enabling highly specific and controlled conditions for covalent closure of the peptide. By utilizing a variety of different strategies for restricting the conformation of these peptides, distance geometries and orientation of the folded peptide can be controlled. Any such strategies employing chemical reactions known in the art may be used.

Using these techniques, synthetic peptide analogs can be made and tested for their abilities to mimic the biological functions of the parent proteins (members of the Ig superfamily.) Generally, these biological functions are protein binding. However, the binding itself can be assayed indirectly, for example, by assaying the biological sequelae to binding. For example, in the case of CD4, the inhibition of HIV-infection can be used to monitor protein binding of CD4 (or its peptide analogs) to gp120 of HIV-1.

Two particularly useful peptide analogs have been found using the techniques described. One comprises amino acids 84-100 of CD4 and one comprises amino acids 42-54 of CD4, representing the CDR3 and CDR2 subdomains, respectively. These peptides have been restricted conformationally using cysteine-cysteine disulfide bonds, but other restricting means may be used advantageously. Both peptides inhibit HIV-1 virus binding to CD4-positive cells. Inhibition of HIV-induced syncytia formation or infectivity can also be used to assess CD4 biological activity and protein binding. Methods of assaying these functions are known in the art.

The present peptides are relatively short in length and therefore they are easily synthesized by chemical m ans. Such synthetic peptides have many advantages over the use of the entire CD4 protein or large portions of the CD4 protein as has been described in the literature. For instance, th large porti ns of the CD4 protein cannot conveniently be made by synthetic techniques and must be made by recombinant DNA techniques which are expensive and time

consuming. Additionally, the larger CD4 proteins may present solubility and immunogenicity problems when introduced into a patient. Short synthetic peptides are much more soluble and less immunogenic than larger proteins.

As used herein, "peptide" refers to a linear series of no more than about 50 amino acid residues connected to one another by peptide bonds between the alpha-amino groups and carboxy groups of adjacent amino acid residues. Additional covalent bonds between portions of the peptide are also present to restrain the conformation of the molecule, such as amide and disulfide bonds. When used herein, "protein" refers to a linear series of greater than 50 amino acid residues connected one to the other as in a peptide. The term "synthetic peptide" means a chemically derived chain of amino acid residues linked together by peptide bonds that is free of naturally occurring proteins and fragments thereof. The term "immunoglobulin superfamily members" refers to a protein which shares structural homology with immunoglobulin molecules. The hallmarks of superfamily members are two beta sheets sandwiching a hydrophobic interior where each sheet is comprised of repeating units of antiparallel beta strands. (See Williams and Barclay (1988), Ann. Rev. Immunol., vol. 6, pp. 381-405.)

The three-letter symbols used to represent the amino acid residues in the peptides of the present invention are those symbols commonly used in the art. The amino acid residues are preferred to be in the "L" isomeric form. However, residues in the "D" isomeric form may be substituted for any L-amino acid, as long as the desired functional property of HIV-1 binding is retained by the peptide. The three-letter symbols used herein refer to the following amino acids: Ser is serine; Il is isol ucine; Gln is glutamine; Ph is phenylalanine; His is histidine; Trp is tryptophan; Lys is lysine; Asn is asparagine; Leu is leucine; Gly is glycine; Thr is threonine; Asp is aspartic acid; Arg is arginine; and Ala is alanine.

Peptides of the present inv ntion include any analog, fragm nt or chemical derivative of the peptides capabl of binding to a protein to which the parent protein binds. For example, one set of peptides

binds to the protein gp120, as does the parent protein, CD4. The term "analog" refers to any peptide having a substantially identical amino acid sequence to the peptides of the invention in which one or more amino acids have been substituted with other amino acids; the substituted amino acids allow or require the peptide to assume the equilibrium conformation of the domain of the parent protein. Often cysteine, lysine, and glutamic acid will be used for their side chains which can form covalent linkages to restrict the conformation of a peptide. In addition, conservative amino acid changes may be made which do not alter the biological function of the peptide. For instance, one polar amino acid, such as glycine or serine, may be substituted for another polar amino acid; or one acidic amino acid, such as aspartic acid may be substituted for another acidic amino acid, such as glutamic acid; or a basic amino acid, such as lysine, arginine or histidine may be substituted for another basic amino acid; or a non-polar amino acid, such as alanine, leucine or isoleucine may be substituted for another non-polar amino acid.

The terms "analog" shall also include any peptide which has one or more amino acids deleted from or added to an amino acid sequence of a parent protein of a superfamily member, but which still retains a substantial amino acid sequence homology to these peptides as well as protein binding ability. A substantial sequence homology is any homology greater than 50% but preferably greater than 90%. The term "fragment" shall refer to any shorter version of the peptides identified herein having at least five amino acid residues, wherein the fragment is capable of binding to the protein to which the parent protein binds.

The peptides of the present invention may be prepared by any of the following known techniques. Conv niently, th peptides may be prepared using the solid-phase synthetic technique initially described by Merrifield, in J. Am. Chem. Soc. 15:2149-2154 (1963). Other peptide synthesis techniques may be found, for example, in M. Bodanszky et al., Peptide Synthesis, John Wiley & Sons, 2d Ed., (1976) as well as in other reference works known to those skilled in the art. A summary of peptide synthesis techniques may be found in J. Stuart

and J. D. Young, Solid Phase Peptide Synthesis, Pierce Chemical Company, Rockford, IL (1984). The synthesis of peptides by solution methods may also be used, as described in The Proteins, vol- II, 3d Ed., Neurath, H. et al., Eds., p. 105-237, Academic Press, New York, NY (1976). Appropriate protective groups for use in such syntheses will be found in the above texts as well as in J. F. W. McOmie, Protective Groups in Organic Chemistry, Plenum Press, New York, NY (1973). Of course, the present peptides may also be prepared by recombinant DNA techniques, although such methods are not preferred because of the need for purification and subsequent chemical modifications to conformationally restrain the peptides.

In general, these synthetic methods involve the sequential addition of one or more amino acid residues or suitably protected amino acid residues to a growing peptide chain. Normally, either the amino or carboxyl group of the first amino acid residue is protected by a suitable, selectively removable protecting group. A different, selectively removable protecting group is utilized for amino acids containing a reactive side group such as lysine.

Using a solid phase synthesis as an example, the protected or derivatized amino acid is attached to an inert solid support through its unprotected carboxyl or amino group. The protecting group of the amino or carboxyl group is then selectively removed and the next amino acid in the sequence having the complementary (amino or carboxyl) group suitably protected is admixed and reacted under conditions suitable for forming the amide linkage with the residue already attached to the solid support. The protecting group of the amino or carboxyl group is then removed from this newly added amino acid residue, and the next amino acid (suitably protected) is then added, and so forth. After all the desired amino acids have been linked in the proper sequence any remaining terminal and side group protecting groups (and solid support) ar removed sequentially or concurrently, to provide the final peptide. The peptides of the invention are devoid of benzylated or methylbenzylated amino acids. Such protecting group moieties may be used in the course of synthesis, but they are removed before the peptides are used. Additional reactions may be

necessary, as described elsewhere to form intramolecular linkages to restrain conformation.

The peptides of the present invention generally contain at least five amino acid residues and up to fifty amino acid residues, preferably between 6 and 20 amino acid residues. These peptides may be linked to an additional sequence of amino acids either or both at the N-terminus and at the C-terminus, wherein the additional sequences are from 1-100 amino acids in length. Such additional amino acid sequences, or linker sequences, can be conveniently affixed to a detectable label or solid matrix, or carrier. Labels, solid matrices and carriers that can be used with peptides of the present invention are described below. Typical amino acid residues used for linking are tyrosine, cysteine, lysine, glutamic acid and aspartic acid, or the like.

The synthetic peptides of the present invention are generally antigenic rather than immunogenic. That is, the peptides may be bound by an antibody specific to them (antigenic) but they may not induce antibody production in a host (immunogenic). Standard techniques that are well known in the art may be used to render the present peptides immunogenic if necessary (that is, if they are not immunogenic in the host as synthesized). One such method is to conjugate the peptide to carrier and then inject the conjugate in effective amount as an immunogenic inoculum into a host.

Any peptide of the present invention may be used in the form of a pharmaceutically acceptable salt. Suitable acids which are capable of forming salts with the peptides of the present invention include inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, phosphoric acid and the like; and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, mal nic acid, succinic acid, maleic acid, fumaric acid, anthranilic acid, cinnamic acid, naphthalene sulfonic acid, sulfanilic acid or th like.

Suitabl bases capable of forming salts with the peptides of the present invention includ inorganic bases such as sodium hydroxide, ammonium hydroxide, potassium hydroxide and the like; and organic

bases such as mono-, di- and tri-alkyl and aryl amines (e.g. triethylamine, diisopropyl amine, methyl amine, dimethyl amine and the like) and optionally substituted ethanolamines (e.g. ethanolamine, diethanolamine and the like).

For use in a method of treating diseases, such as AIDS and autoimmune diseases, the synthetic peptides of the present invention may be present in a pharmaceutical composition in admixture with a pharmaceutically-acceptable carrier. The pharmaceutical composition may be compounded according to conventional pharmaceutical formulation techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g. sublingual, rectal, nasal, oral or parenteral. Compositions for oral dosage form may include any of the usual pharmaceutical media, such as, for example, water, oils, alcohols, flavoring agents, preservatives, coloring agents and the like in the case of oral liquid preparations (e.g. suspensions, elixirs and solutions) or carriers such as starches, sugars, diluents, granulating agents, lubricants, binders, disintegrating agents, and the like in the case of oral solid preparations (e.g. powders, capsules and tablets). Controlled release forms may also be used. Because of their ease in administration, tablets and capsules represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are obviously employed. If, desired, tablets may be sugar coated or enteric coated by standard techniques.

For compositions to be administered parenterally, the carrier will usually comprise sterile water, although other ingredients to aid solubility or for preservation purposes may be included. Injectable suspensions may also be prepared, in which case appropriate liquid carriers, suspending agents and the like may be employed. The parenteral routes of administration may be intravenous injection, intramuscular injection or subcutaneous injection.

Th pharmaceutical compositions of the present invention may be used, for example, in a method for treating a patient suspected of being infected with HIV-1 or HIV-2. The method comprises administering to the patient an effective, HIV-1 or HIV-2-neutralizing amount

peptides in th composition will bind to the gp120 of HIV-1 or HIV-2 and prevent the HIV-1 or HIV-2 from binding to the CD4 receptor on susceptible T-cells. Such interruption of the binding process will prevent the T4 cell from being infected by the HIV-1 or HIV-2 and will thereby prevent the usual immunosuppression observed in an HIV-1 or HIV-2-infected patient. Other peptides of the invention in pharmaceutical compositions can be used to inhibit binding of other viruses or autoantibodies to their receptors or target cells. Such inhibition can prevent infection, inhibit spread of infection, prevent autoimmune destruction of body tissues, etc.

The present invention also contemplates antibodies which are specific for the peptides of the present invention. It is believed that some such antibodies will prevent the binding of the HIV-1 or HIV-2 to the CD4 receptor of the T4 cell, for example. Such antibodies will bind to the CD4 protein and thereby inhibit the binding of the HIV-1 or HIV-2 to that protein. Such antibodies may be polyclonal or monoclonal; however, it is preferred that the antibodies be monoclonal.

Polyclonal antibodies may be prepared according to methods well known in the art, such as those described in Benedict, A. A., et al., "Production of Antiserum", Methods in Immunology 1:197-306 (1967). It is preferred that monoclonal antibodies be used in the present method because they are specific for a single epitope and therefore provide more reproducible results. Methods of producing monoclonal antibodies are well known in the art, such as the well known Kohler and Milstein method. This method as well as variations thereof may be obtained by reference to Yelton, D. E. et al., Ann. Rev. of Biochem. (1981) 50:657-80.

The present invention also contemplates an anti-idiotype antibody that bears an internal image of a peptid of the present invention and which is thereby capable of binding to a protein to which the parent superfamily member binds. Such an anti-idiotype antibody may be used in any th rapeutic or diagnostic format where th formation of an immunoreaction product containing the protein to which a [illegible] member binds is desired. For example, an

anti-idiotype antibody which has the internal imag of a CDR3 domain could be used to diagnostically detect the HIV-1 or HIV-2 virus in serum samples.

By way of background, a brief description of anti-idiotype antibodies is provided. The immune system of an individual is capable of producing millions of different types of antibodies. Each antibody can in turn be the target of other antibodies that recognize its unique molecular characteristics. By means of such antibody-antibody reactions, the immune system interacts with itself. These interactions are known as idiotype-anti-idiotype reactions. In such reactions, an initial antibody (Ab-1) that is isolated from an animal serum or hybridoma supernatant, for example, is injected into another animal and induces the formation of a second antibody (Ab-2). The Ab-2 is specific for, and binds to, the Ab-1 and is said to recognize Ab-1's paratope or idiotope. The Ab-2 produced in response to the idiotope is referred to as an anti-idiotype antibody. Thus, an anti-idiotype antibody may be defined as an antibody that is capable of reacting with an Ab-1 elicited by a single antigen. For more complete background information on anti-idiotype antibodies, see Kennedy, R.C. et al. "Anti-Idiotypes and Immunity," Scientic American, pages 48-56 (July 1986).

Idiotopes are antigenic determinants (epitopes) expressed by an antibody molecule. Internal image idiotopes are antigenic determinants expressed by an antibody molecule that are immunologically similar or identical to antigenic determinants found on an antigen that is foreign or external to the immune system. The operational theory explaining the immunologic similarity of an internal image idiotype and its corresponding external antigenic determinant is that the internal image idiotype is a conformational homolog f the external antigenic determinant. Because they are conformationally homologous, an internal image idiotype and its corresponding external antigenic determinant are immunologically isoreactive, i.e., an antibody induced by on will immunoreact with th other. Therefore, an

peptide of the present invention (an external antigenic determinant) can be substituted for that peptide in immunochemical reactions.

The production of an antibody expressing an internal image corresponding to an antigenic determinant on a protein requires finding or producing an immunogenic "template" that can induce the internal image bearing antibody. Templates for producing internal image antibodies are immunogenic molecules having at least a portion of their conformation that is complimentary to or the "mirror image of" the antigenic determinant of interest. Template-bearing molecules used by the art to induce internal image-bearing antibodies are proteins that bind the protein of interest. Such proteins include antibody molecules whose antibody combining sites immunoreact with the protein of interest as well as cell surface receptor proteins which can bind the protein of interest. In either case, the critical feature of the binding protein is that portion of its structure that interacts with the protein of interest because it is only that portion that can act as a template. Thus, the antibodies of the present invention specific for the peptides of the present invention can be used as immunogens to induce an anti-idiotypic antibody. A particular preferred anti-idiotypic antibody will have that idiotope that can block the CD4 binding site on gp120 of the HIV-1 or HIV-2.

It is preferred that the anti-idiotype antibody be raised against a monoclonal antibody. The preparation of an anti-idiotypic antibody against a monoclonal antibody combining site is well known in the art. For example, see Staudt et al., J. Exp. Med., 157:687-704 (1983); and Regan et al., J. Virol. 41:660-666 (1983). Briefly, to produce an anti-idiotypic antibody composition of this invention, a first laboratory mammal is inoculated with an immunologically-effective amount of a synthetic peptide of the present inv ntion, typically as present in a vaccine of the present invention. The anti-peptide antibody molecules thereby induced are then collected from the first mammal and those immunospecific for the peptide are isolated to the extent desired by well known techniques such as, for example, by immunoaffinity chromatography. This provides the Ab1 preparation. The Ab1 preparation is used to inoculate a second laboratory mammal

to produce an Ab2. Those antibodies produced by the second mammal which are immunospecific for both the Ab1 and the protein to which the superfamily member and peptide bind are isolated to the extent desired by techniques will known in the art.

Monoclonal anti-idiotypic antibody compositions are contemplated by the present invention. A monoclonal anti-idiotypic antibody composition contains, within detectable limits, only one species of antibody combining site capable of effectively binding both the Ab1 and the protein to which the peptide and superfamily member binds. Thus, a monoclonal anti-idiotypic antibody composition of the present invention typically displays a single binding affinity for its binding partner.

In a preferred embodiment, it is contemplated that the Ab1 antibodies of the present invention can block the binding site on the CD4 receptor so that the HIV-1 or HIV-2 virus cannot infect the T4 cell. Therefore, these Ab-1 antibodies may be used in a passive vaccine to confer protection on a patient against HIV-1 or HIV-2. Such a vaccine may also be helpful in preventing maternal transmission of virus to neonates. Additionally, such a vaccine may function as an active vaccine by stimulating the patient to produce anti-idiotype antibodies in response to the Ab-1 antibody. Accordingly, a vaccine of the present invention may comprise an effective immunizing amount of an Ab1 antibody of the present invention in a pharmaceutically-acceptable carrier. It is preferred that the antibody used in the vaccine is a monoclonal antibody. "Humanized" monoclonal antibodies are preferred as they will be less immunogenic than a murine monoclonal, for example. Such humanized antibodies include portions of human antibodies in their structures.

Ab2 molecules can also be used as a vaccine. Ab2 molecules can be used to bind to the protein to which the superfamily member binds, thereby inhibiting the physiologic binding of that protein t the superfamily member itself.

The antibody molecule may be operatively linked to an immunogenic carrier. As used herein, the term "operatively linked" means

linking means that does not interfere with the ability of either of the linking groups to function as described. Because immunogenic carriers are typically proteins themselves, the techniques of protein conjugation or coupling through activated functional groups is particularly applicable. For review of those techniques, see Aurameas et al., Scand. J. Immunol., Vol. 8, Supp. 1, pp. 7-23 (1978). See, also, U.S. Patent Nos. 4,493,795 and 4,671,958. Useful immunogenic carriers are well known in the art and are generally large proteins. Exemplary of such carriers are keyhole limpet hemocyanin (KLH); edestine, thyroglobulin; albumins, such as bovine serum albumin (BSA) or human serum albumin (HSA); red blood cells, such as sheep erythrocytes (SRBC); tetanus toxoid; cholera toxoid; and polyamino acids, such as poly(d-lycine:d-glutamic acid) and the like.

The vaccine may also contain an immunopotentiator, which is a molecular entity that stimulates the maturation, differentiation and function of B and/or T-lymphocytes. Immunopotentiators are well known in the art and include T-cell stimulating polypeptides such as those described in U.S. Patent No. 4,426,324 and the C8-substituted guanine nucleosides described by Goodman et al., J. Immunol., 115:3284-3288 (1985) and U.S. Patent No. 4,643,992. The phrases "suitable for human use" and "pharmaceutically-acceptable" refer to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human.

The preparation of a vaccine that contains an antibody molecule as an active ingredient is well understood in the art. Typically, such vaccines are prepared as injectibles, either as liquid solutions or suspensions; solid forms suitable for solution in or suspension in liquid prior to injection may also be prepared. The preparation may also be emulsified.

The active immunogenic ingredient may be dissolved, dispersed or admixed in an excipient that is pharmaceutically-acceptable and compatible with the active ingredient as is well known. Suitable excipients are, for xample, water, saline, phosphate buffered saline (PBS), dextrose, glycerol, ethanol or the like and combinations thereof. In addition, if desired, the vaccine may contain minor

amounts of auxiliary substances such as wetting or 35 emulsifying agents, pH buffering agents or adjuvants which
enhance the effectiveness of the vaccine.

The vaccines may conventionally be administered parenterally, by either intravenous, subcutaneous, or intramuscular injection. Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral formulations. For suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, cellulose, magnesium carbonate and the like. Compositions may take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders. The anti-peptide antibody can be formulated into a vaccine as a neutral or salt form. Pharmaceutically-acceptable salts include those listed above.

The vaccines may be administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective and immunogenic. The quantity to be administered depends on the subject to the treated, capacity of the subject's immune system to synthesize the antibodies and the degree of protection desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual.

The vaccine may also include an adjuvant as part of the excipient. Adjuvants such as complete Freund's adjuvant (CFA) or, incomplete Freund's adjuvant (IFA) for use in laboratory animals are well known in the art. Pharmaceutically-acceptable adjuvants such as alum may also be used.

The present invention also contemplates pharmaceutical compositions which comprise one of the peptides of the present invention and an antiviral agent as a form of combination therapy for HIV-1 or HIV-2 infection. The peptide and antiviral agent may be in separate m lecular form or they may be linked or conjugated by a chemical bond. In the case where the peptide and antiviral agent are linked

the resulting conjugated molecule would serve as a means for specific targeted delivery of the antiviral agent to the HIV-1 or HIV-2. It is also envisioned that the peptide and antiviral agent may be administered separately in a combination therapy. Fischer et al. (Nature 331:76-7815 (1988)) suggested the use of an antiviral therapy for HIV-1 or HIV-2 which involves administering to a patient soluble CD4 protein in combination with an antiviral drug that blocks reverse transcriptase. In this scenario the two therapeutic agents are not linked and need not be physically admitted.

The antiviral agent may be any agent that is capable of blocking one or more stages in the life cycle of HIV-1 or HIV-2. One class of such an antiviral agent is a reverse transcriptase inhibitor which is capable of inhibiting the function of the enzyme reverse transcriptase present in all retroviruses. Many of the reverse transcriptase inhibitors presently in use are nucleoside analogs or derivatives, such as 3'-azido-3'-deoxythimidine (AZT); dideoxycytidine; dideoxyadenosine and other nucleoside analogs, such as acyclovir, ribavirin, and vidarabine. European Patent Application Publication No. EP 196,185 describes the use of AZT for treating AIDS. These nucleoside analogs interrupt the reverse transcriptase as it assembles the viral DNA destined to become the pro-virus of HIV-1 or HIV-2. The antiviral drugs used for this purpose are typically chemical analogs of the nucleosides that form the sub-units of DNA. When the analog is supplied to an infected cell, reverse transcriptase will incorporate it into a growing DNA chain. Because the analog lacks the correct attachment point for the next sub-unit, however, the chain is terminated. The truncated DNA cannot integrate itself into the chromosomes or provide the basis f r viral replication, and, so, th spread of infection is halted.

Other anti-viral agents that may be useful are those which can block the formation of the HIV-1 or HIV-2 envelope protein, such as castanospermine; any agent which can interfere with the assembly of new virus components in infected cells; or any agent which can inter-

It is also contemplated that the peptides of th present invention may find use as diagnostic agents for detecting the presence of the proteins to which a superfamily member binds, such as gp120 of HIV-1 or HIV-2. Such a diagnostic system in kit form includes, in an amount sufficient for at least one assay, a peptide or anti-idiotype antibody composition of the present invention, as a packaged reagent. Instructions for use of the packaged reagent are also typically included in such a kit. The peptide or anti-idiotypic antibody may be labeled with a detection means such as a radioactive, fluorescent or enzymatic label. Such a detection means allows for the detection of the peptide or anti-idiotype antibody when it is bound to its binding partner. The peptide or anti-idiotype antibody may be used as a "capture ligand" bound to a solid matrix. In such a case, a labelled antibody specific for the binding partner is used to detect the binding partner which was captured by the ligand. Alternatively, the peptide or anti-idiotype antibody may be used as the "detector ligand" and an antibody specific for the binding partner can used as the capture ligand.

As used herein, the term "packaged" refers to a solid matrix for materials such as glass, plastics, paper, foil and the like capable of holding, within fixed limits, a peptide or anti-idiotypic antibody composition of the present invention. Thus, for example, a package can be a glass vial used to contain milligram quantities of a contemplated peptide or anti-idiotypic antibody or it can be a microtiter plate well to which microgram quantities of a contemplated peptide or anti-idiotypic antibody have been operatively affixed, i.e., linked so as to be capable of specifically binding its binding partner. "Instructions for use" typically include a tangible expression describing the reagent concentration or at least one assay method parameter such as the relativ amounts of reagent sample to be admixed, maintenance time periods for reagent/sample admixtures, temperature, buffer c ndi-tions and the like.

As used herein, the t rms "label" and "detector means" and their various grammatical forms refer to single atoms and molecules that are either directly or indirectly inv lved in the production of a

detectabl signal to indicate the presence of a complex. The word "complex" as used herein refers to the product of specific binding reactions, such as between the peptide and the protein to which a superfamily member binds or between the peptide and an antibody specific for it. Any label or detection means can be linked to or incorporated into the peptide or anti-idiotypic antibody of the present invention, or used separately, and those atoms or molecules can be used alone or in conjunction with additional reagents. Such labels are themselves well known in clinical diagnostic chemistry and constitute a part of this invention only insofar as they are utilized with otherwise novel peptides and anti-idiotype antibodies.

The labeling means can be a fluorescent labeling agent that chemically binds to antibodies or antigens without denaturing them to form a fluorochrome (dye) that is a useful immunofluorescent tracer. Suitable fluorescent labeling agents are fluorochromes, such as fluorescein isocyanate (FIC), fluorescein isothiocyanate (FITC) and the like. A description of immunofluorescence analysis techniques is found in DeLuca, "Immunofluorescence Analysis", in Antibody as Tool, Marchalonis et al., Eds., John Wiley & Sons Limited, pp. 189-231 (1982).

In preferred embodiments, the indicating group is an enzyme, such as horseradish peroxidase (HRP), glucose oxidase or the like. In such cases where the principal indicating group is an enzyme such as HRP or glucose oxidase, additional reagents are required to visualize the fact that a binder complex has formed. Such additional reagents for HRP include hydrogen peroxide and an oxidation dye-precursor, such as diaminobenzidine.

Radioactive elements are also useful labeling agents and are used illustrativ ly herein. An exemplary radio-labeling agent is a radioactive element that produces gamma ray emissions. Elements which emit gamma rays, such as ^{124}I, ^{125}I, ^{128}I, ^{132}I and ^{51}Cr repres nt one class of gamma ray emission-producing radioactive el ment indicating groups. Particularly preferred is ^{125}I. Another group of useful labeling means are those elements such as ^{11}C, ^{18}F, ^{15}O and ^{13} [illegible] positrons. The positrons so emitted

produce gamma rays upon encounter with electrons present in th test solution.

The labeling of proteins is well known in the art. For instance, antibody molecules produced by a hybridoma can be labeled by metabolic incorporation of radioisotope-containing amino acids provided as a component in the culture medium. See, for example, Galfre et al., Meth. Enzymol, 11:3-46 (1981). The techniques of protein conjugation or couplings through activated functional groups are particularly applicable.

The diagnostic kits of the present invention may be used in a solid phase format to detect the presence of a quantity of, for example, an infectious agent such as HIV-1 or HIV-2, in a body fluid sample such as serum, plasma or urine. In the solid phase assay, the peptide or anti-idiotype antibody may be bound to a solid phase and then contacted with the sample containing the HIV-1 or HIV-2. The HIV-1 or HIV-2 in the sample will bind to the peptide or anti-idiotype antibody. Thus, in preferred embodiments, a peptide or anti-idiotype antibody molecule composition of the present invention may be affixed to a solid support to form a solid support that comprises a package in the subject diagnostic system. The reagent is typically affixed to the solid matrix by adsorption from an aqueous medium, although other modes of affixation, well known to those skilled in the art, can be used. Useful solid matrices are also well known in the art. Such materials are water-insoluble and include the cross-linked dextran available under the trademark SEPHADEX™ from Pharmacia Fine Chemicals; agarose; beads of polystyrene; polyvinylchloride; polystyrene; cross-linked polyacrylamide; nitrocellulose- or nylon-based webs such as sheets, strips or paddles; or tubes, plates or the wells of microtiter plates such as those made from polystyren or polyvinylchloride. These same solid matrices to which the peptid or antibody of the invention has been bound can also be used preparatively. That is, they can be used to prepare purified or concentrated preparations comprising the protein or agent to which the peptide or antibody binds. Alternatively, the matrices can be used to remove the

certain proteins or agents by incubation with the matrices, and collecting the portion which does not bind to the matrix. In the case of concentration or purification of the protein or agent which binds to the agent, the non-binding portion is removed and discarded and the binding portion is eluted by means known in the art.

The peptide or anti-idiotype antibody of the present invention can be used in the diagnostic system in solution form, as a liquid dispersion, or as a substantially dry powder, e.g. in lyophilized form. Where the indicating means is an enzyme, the enzyme substrate can also be provided in a separate package of the system. A solid support such as the before-described microtiter plate and one or more buffers can also be included as separately packaged elements in this diagnostic assay system.

Throughout this disclosure, various publications and patents are referenced. The disclosure of these publications and patents in their entireties are hereby incorporated by reference into this disclosure in order to more fully describe the state-of-the-art as known to those skilled therein as of the date of the invention described and claimed herein. The invention has been described herein with reference to certain preferred embodiments and examples. However, since obvious variations will appear to those skilled in the art, the invention is not to be considered limited thereto. The invention is exemplified by the following experimental work. The invention is not limited by these examples but is defined by the specification as a whole.

Example 1

This example demonstrates homology modeling of the V_1 domain of the CD4 protein according to the light chain variable domain of a human immunoglobulin.

The V_1 domain of th CD4 protein (residues 1-104) was modeled based on the high resolution crystallographic structure of th light chain variable domain of a human Fab homodimer immunoglobulin fragment [REI] (Epp et al., 1975). The REI structure was utilized because it displayed the highest degree of ov rall h mology with CD4 f all of the known Ig structure compared. It should be noted that the homology between the CDR3 region of REI is poor when compared to

the equivalent region of the CD4 protein (other known Ig structures have greater degrees of homology in the CDR3 region than REI). In modeling experiments conducted in our laboratory using known Ig structures, however, we have observed that our most consistent results were obtained when complete structures were used as templates, rather than utilizing pieces of different structures based on highest local homology.

The amino acid composition of the CD4 protein (residues 1-104) was superimposed on the REI backbone and the newly incorporated side chains were allowed to orient themselves in a successive series of energy minimization calculations. Because a generic Ig-fold consists of a repetitive series of paired anti-parallel beta strands, we initially treated these paired substructures as elemental units. In the first series of dynamic calculations, the anti-parallel strands were allowed to realign themselves, subsequently the paired strands were allowed to interact with neighboring strands and finally the entire structure was allowed to conformationally equilibrate. The resulting structure is stable in long dynamic runs (greater than 50 picoseconds) and is stable to energy pertubations such as heating and cooling. The resulting structure is shown in Figure 3.

The CD4 model generated in this fashion agrees well with all known published observations of the protein and, more importantly, has proven to be an effective template in the design of conformationally-restricted synthetic peptides (see below). Examination of this structure shows that there is a CDR1-like domain spanning CD4 residues 16-28, a CDR2-like domain spanning residues 44-60 and a CDR3-like domain spanning residues 84-101. This model also indicates that the CDR1-, CDR2- and CDR3-lik regions of the CD4 protein fold tog ther t form a continuous surface comprised of residues 21-27 (CDR1), 39-42 (part of a framework regi n), 46-53 (CDR2), 85-88 and 92-98 (both from CDR3).

Example 2

This example demonstrates the selection and synthesis of

Our modeled CD4 structure was used as a design template in the construction of our synthesized analogs. According to our modeled structure, the CDR3 region of the CD4 protein initiates at residue 84(Cys) and terminates at residue 101(Thr). In order to conformationally restrict the folding equilibria of the resulting synthetic peptides, we examined the CDR region for residues in the base of the anti-parallel beta strands that are naturally separated by the approximate distance of a disulfide bond (~6 A). Using computer-assisted modeling, cysteine residues were substituted in these positions and the predicted folding patterns of the putative structures were tested for their ability to mimic the structure observed in our model of the parent V_1 domain. The residues marking the beginning and the end of the CDR3-like domain, residues 84 and 101, met the criteria described above.

Two peptides were synthesized according to the solid phase procedures outlined by Jameson et al. (1988). The first peptide incorporated the native CD4 residues 84-101, while in the second peptide residue 101(Thr) was substituted with a cysteine residue, such that cysteines were present at each end of the peptide. Reverse phase HPLC-purified peptides were used in all of the assays described below.

In addition to the two CDR3 peptides described above, five peptides were synthesized from the CDR2-like domain with artificial constraints introduced in the form of cysteine residues. See Figure 1.

Example 3

This example demonstrates the biological activities of the peptide analogs.

The most potent virus binding inhibitor among the CDR-2 analogs was the 45-54 peptide, with cysteine residues artificially introduced in position 45 and 54. This peptide was able to inhibit virus binding in a FACS-type binding assay (McDougal et al., 1986; see experimental design section) down to concentrations f 50μg/ml (~50μM). This observation is consistent with the genetic mutational data which shows that gp120 binding is abolished by mutations in this region. According to our V_1 domain model, this region, residues

the three CDR domains. It should also be noted that in the experiments with the CDR2-derived peptides that all of the peptides have been purified and used directly as heterogeneous mixed disulfide populations.

The CDR3 peptide analog from residues 84-101 with cysteines at position 84 and 101 is a potent inhibitor of virus binding. The peptide with the native CD4 amino acids (thr at position 101) was devoid of activity in the virus binding inhibition assay up to a concentration of 1 mg/ml.

Example 4

This example shows that the peptide analog, and not derivatized by-products, causes inhibition of HIV binding.

Reverse phase HPLC purified versions of the "84-101" peptide analog retain the entire activity, thus making it unlikely that derivatized by-products are responsible for the observed activity. In order to further establish that the parent peptide analog was responsible for the binding inhibition, we performed crude refolding experiments. The peptide was purified with low pH solvents (containing 0.1% trifluoroacetic acid) so that disulfide bridge formation was not favored. After lyophilization, the peptide was diluted to 20 μg/ml at 4°C and allowed to refold for 5 days in the presence of O_2. The resulting peptide is referred to as refolded. It should be noted that our refolded peptide is still comprised of mixed disulfides, however, the HPLC peak corresponding to intramolecular disulfide formation has been enriched (data not shown).

We have also synthesized the equivalent peptide from the mouse CD4 protein (the L3T4 protein). The mouse peptide was also subjected to our crude refolding procedure. The results of assays of biological activity of these peptides is shown in Figure 2.

These results indicat that the human 84-101 peptide analog shows conformational and concentration dependent inhibition of HIV binding. Were the inhibition due to a synthetic cleavage by-product (as was observed by Lifson et al. (1988)), then one would not expect to see inhibition enhanc ment upon refolding which we have shown leads to enrichment of the intramolecular disulfide-bridged species.

Interestingly, the corresponding mouse 84-101 peptrides also inhibit HIV binding, albeit at significantly greater concentrations. It is likely that the limited mouse/human genetic substitutions in this region of the CD4 protein did not abrogate CD4 binding to gp120 because th mouse structure is significantly similar to that of the human structure

CLAIMS

1. A synthetic peptide comprising an amino acid sequence corresponding to a portion of a member of a family of proteins known as the immunoglobulin superfamily, said member having the ability to bind specifically to a second protein, said peptide having a restricted conformation and having the ability to bind to the second protein, said peptide being free of benzylated and methylbenzylated amino acids.

2. The peptide of claim 1 wherein the peptide is between about 6 and about 20 amino acids in length.

3. The peptide of claim 1 wherein the member is CD4 protein.

4. The peptide of claim 3 wherein the peptide comprises the CDR3 domain.

5. The peptide of claim 3 wherein the peptide comprises the CDR2 domain.

6. The peptide of claim 4 wherein the peptide comprises amino acids 84-100 of CD4.

7. The peptide of claim 5 wherein the peptide comprises amino acids 42-54 of CD4.

8. The peptide of claim 1 wherein the restricted conformation is restricted by means of a cysteine-cysteine disulfide bond.

9. The peptide of claims 1 wherein the restricted conformation is restricted by means of an amide bond formed between side chains of a lysine residue and a glutamic or aspartic acid residue.

10. The peptide of claim 1 wherein the restricted conformation is restricted by means of a toluene 2,4-diisocyanate cross-link between two free amino groups of said peptide.

11. The peptide of claim 1 wherein the restricted conformation is determined using an experimentally determined equilibrium conformation of a second member of the immunoglobulin superfamily, said second member not containing the amino acid sequence of the synthetic peptide.

12. The peptide of claim 7 additionally comprising cysteine

13. Th peptide of claim 6 additionally comprising a cysteine residue in place of amino acid 101 of the CD4 protein.

14. The peptide of claim 3 which inhibits binding of HIV-1 virus to cells expressing the CD4 protein.

15. The peptide of claim 3 which inhibits the infection of cells expressing the CD4 protein by HIV-1 virus.

16. A method of designing a peptide analog to a conformational domain of a member of a family of proteins known as the immunoglobulin superfamily of proteins, comprising:

providing a first model of a conformational domain of a first member of the immunoglobulin superfamily of proteins;

generating by homology modeling a second model of an homologous domain of a second member of the superfamily to form a second model of a molecule having a primary structure corresponding to a portion of the second member;

determining a distance between two parts of the molecule in the second model at conformational equilibrium;

modifying the primary structure of the molecule to restrict the distance between said two parts of the molecule to the determined distance.

17. The method of claim 16 wherein the step of modifying comprises introducing one or more cysteine residues to form an intramolecular disulfide bond.

18. The method of claim 16 wherein the step of modifying comprises introducing an amino acid selected from the group consisting of lysine, glutamic acid or aspartic acid, to the molecule.

19. The method of claim 16 wherein the step of modifying comprises reacting side chains of a lysine and a glutamic or aspartic acid residue to form an amide bond internally cross-linking the molecule.

20. Th method of claim 16 wh rein the step of modifying comprises introducing a toluen 2,4-diisocyanate to internally cross-link two free amino groups of the molecul .

21. Th m thod of claim 16 wherein the first member is a structurally determined member of the Ig-superfamily.

22. The method of claim 16 wherein the first model is of the light chain variable domain of a human Fab homodimer immunoglobulin fragment.

23. The method of claim 16 wherein the model of the first member is obtained by high resolution X-ray crystallography.

24. The method of claim 16 wherein the step of generating a second model by homology modeling comprises adjusting bond lengths.

25. The method of claim 16 wherein the step of generating a second model by homology modeling further comprises realigning anti-parallel beta strand portions of said backbone to form sets of paired strands.

26. The method of claim 25 wherein the step of generating a second model by homology modeling further comprises realigning each set of paired strands relative to said paired strands' nearest neighboring set of paired strands.

27. A method of producing a peptide having a restricted conformation, comprising:

providing a peptide comprising a portion of a protein of a family of proteins known as the immunoglobulin superfamily of proteins;

determining conformational equilibrium of the portion of the protein in the whole protein;

introducing a covalent modification into the peptide to restrict a distance between two parts of the peptide to a distance between the two parts of the peptide in the equilibrium conformation determined.

28. The method of claim 27 wherein the modification comprises a cysteine residue capable of forming an intramolecular cysteine-cysteine disulfide bond.

29. The method of claim 27 wherein the modification comprises a molecule of toluene 2,4-diisocyanate linking two amino groups.

30. The method of claim 27 wherein the modification comprises an amide bond cross-linkng a lysine and a glutamic or aspartic

31. An antibody made by the process of:

immunizing a mammal with a synthetic peptide of claim 1.

32. An antibody made by the process of claim 31 further comprising:

collecting serum of said mammal containing antibody specific for the synthetic peptide.

33. An antibody made by the process of claim 31 further comprising:

immortalizing lymphocytes or spleen cells of said mammal; and

collecting antibodies secreted by the lymphocytes or spleen cells which have been immortalized.

34. An anti-idiotype antibody which specifically recognizes the paratope of the antibody of claim 31.

35. A pharmaceutical composition comprising:

the peptide of claim 1 and a pharmaceutically acceptable carrier.

36. A diagnostic kit comprising:

the peptide of claim 1; and

means for det cting a complex f th peptide bound to the second protein.

37. A composition for purifying blood or serum comprising:

the peptide of claim 1 affixed to a solid matrix.

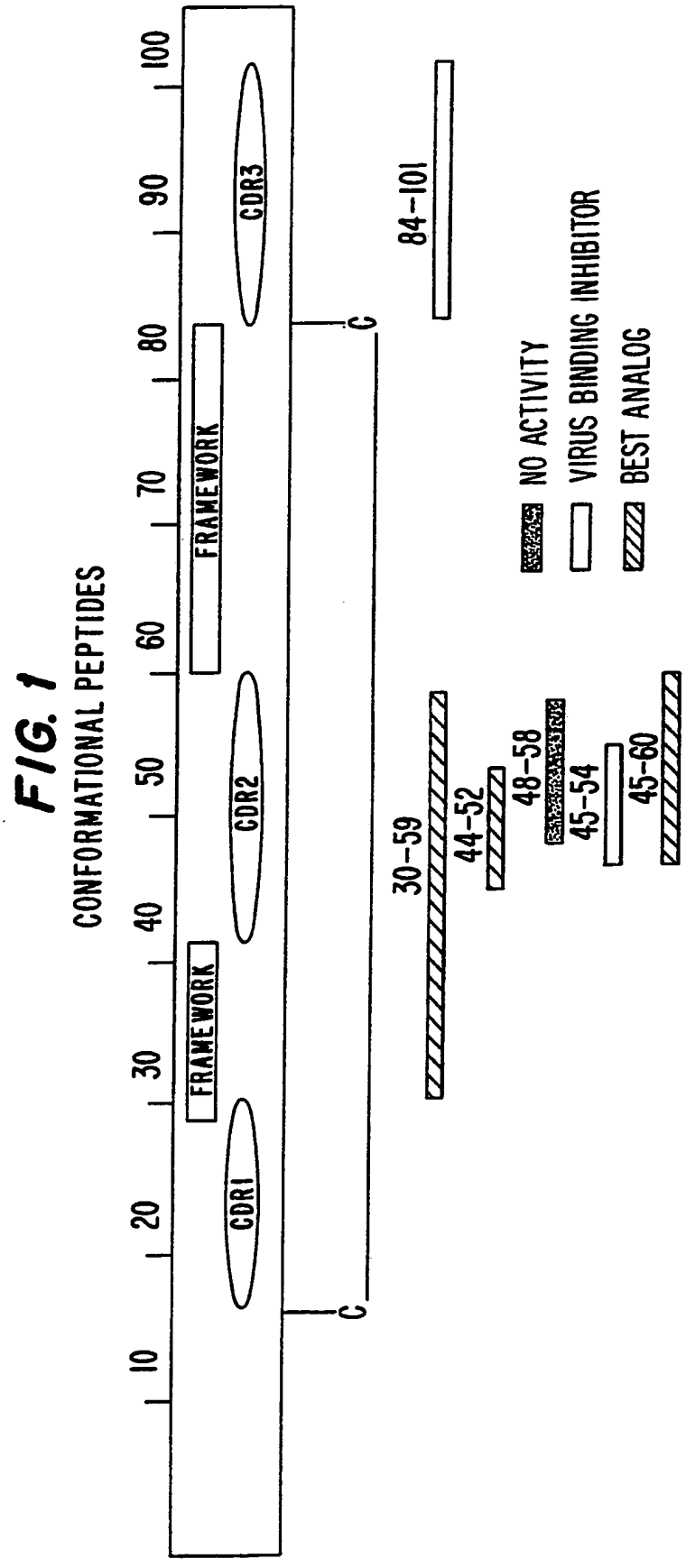


FIG. 1
CONFORMATIONAL PEPTIDES

FIG. 2

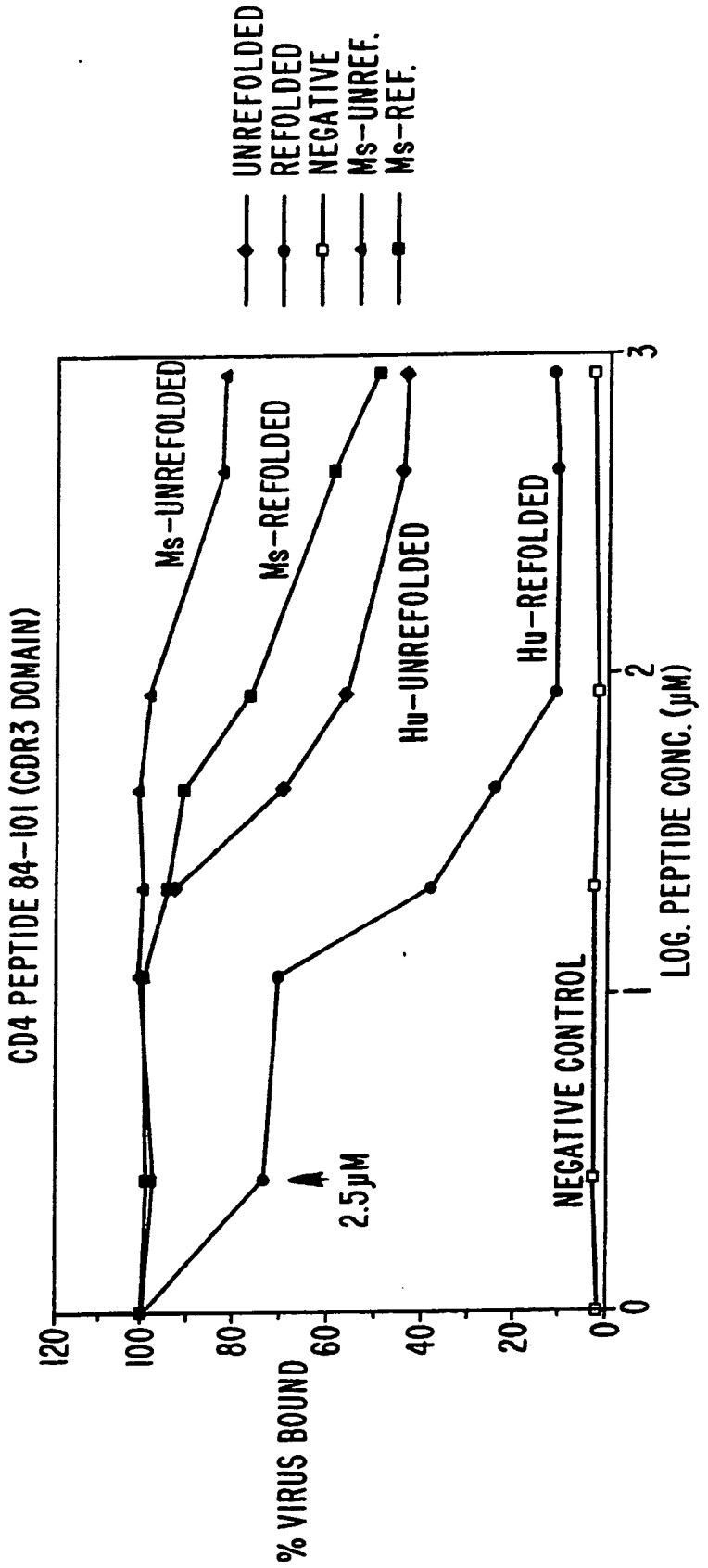
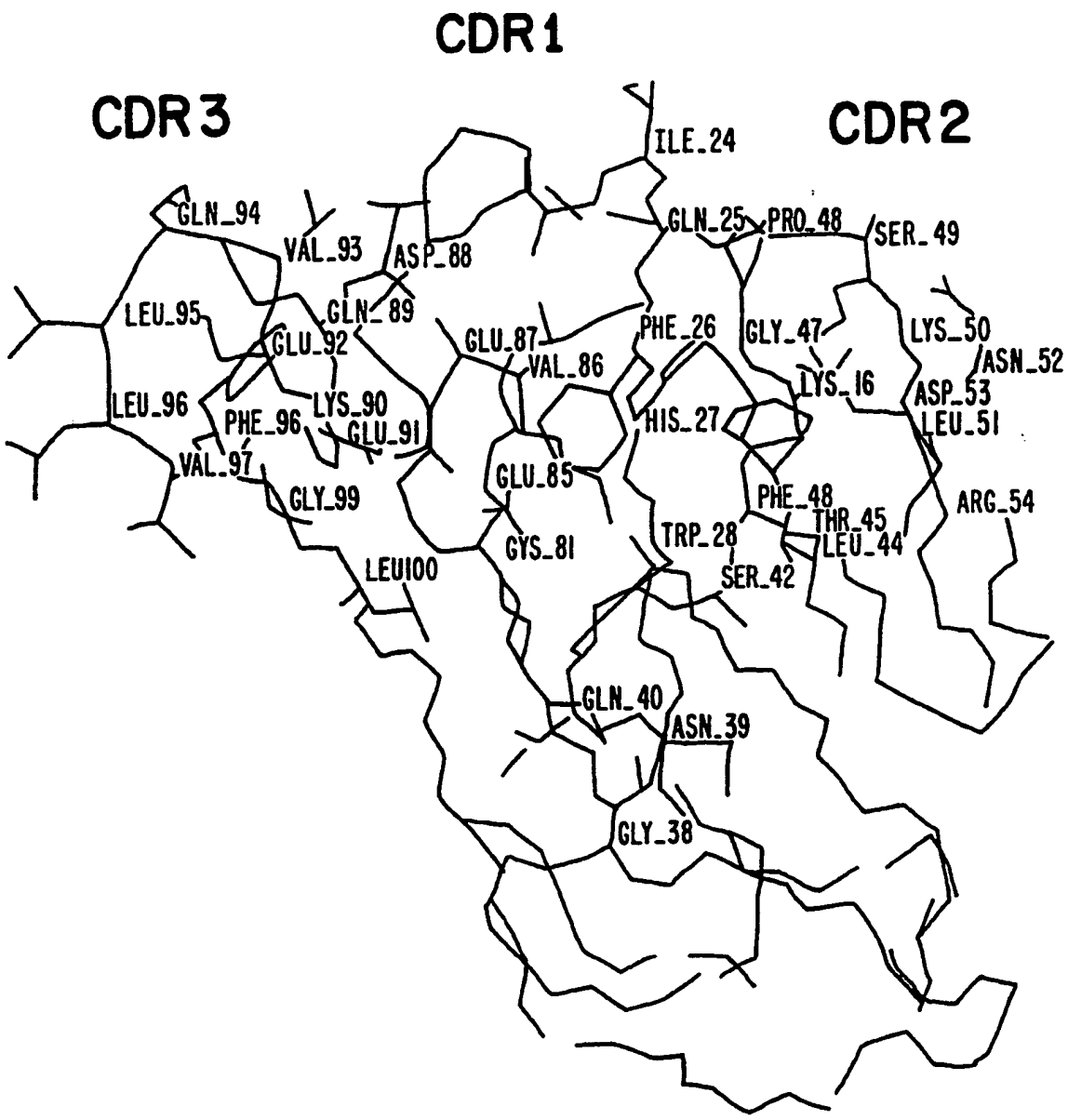

UNREFOLDED
REFOLDED
NEGATIVE
Ms-UNREF.
Ms-REF.
CD4 PEPTIDE 84-101 (CDR3 DOMAIN)
Ms-UNREFOLDED
Ms-REFOLDED
Hu-UNREFOLDED
Hu-REFOLDED
2.5μM
NEGATIVE CONTROL
% VIRUS BOUND
120
100
80
60
40
20
0
0
1
2
3
LOG. PEPTIDE CONC. (μM)

FIG. 3


CDR1
CDR3
CDR2
ILE_24
GLN_94
VAL_93
ASP_88
GLN_25
PRO_48
SER_49
LEU_95
GLN_89
GLU_92
GLU_87
PHE_26
GLY_47
LYS_50
ASN_52
VAL_86
LYS_16
ASP_53
LEU_96
PHE_96
LYS_90
GLU_91
HIS_27
LEU_51
VAL_97
GLU_85
PHE_48
THR_45
ARG_54
GLY_99
TRP_28
LEU_44
GYS_81
SER_42
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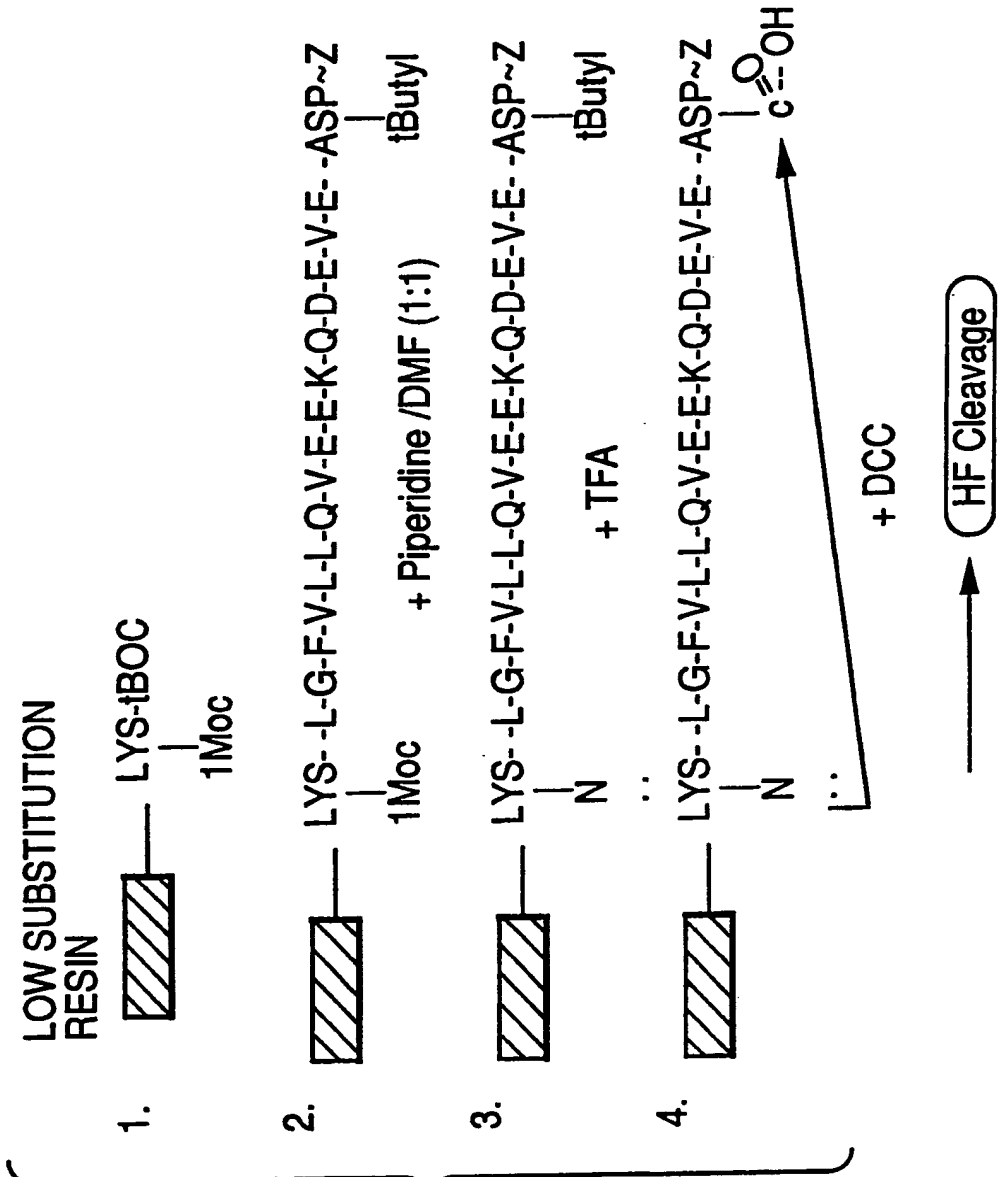
LOW SUBSTITUTION RESIN
1.
LYS-tBOC
1Moc
2.
LYS- -L-G-F-V-L-L-Q-V-E-E-K-Q-D-E-V-E- -ASP~Z
1Moc
tButyl
+ Piperidine /DMF (1:1)
3.
LYS- -L-G-F-V-L-L-Q-V-E-E-K-Q-D-E-V-E- -ASP~Z
N
tButyl
+ TFA
4.
LYS- -L-G-F-V-L-L-Q-V-E-E-K-Q-D-E-V-E- -ASP~Z
N
C=O
OH
+ DCC
HF Cleavage

FIG. 4

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 91/02832

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5	C 07 K 7/04	C 07 K 1/00	C 12 P 21/08
A 61 K 37/02	G 01 N 33/564	G 01 N 33/68	G 01 N 33/569

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	C 07 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	NATURE, vol. 341, 5 October 1989, (London, GB), B.A. JAMESON: "Modelling in peptide design", pages 465-466, see the whole article (cited in the application)	1-30,35-37
X	SCIENCE, vol. 240, 3 June 1988, (Lancaster, PA, US), B.A. JAMESON et al.: "Location and chemical synthesis of a binding site for HIV-1 on the CD4 protein", pages 1335-1339, see the whole article (cited in the application)	1,3,4,14,15,31-37
X	EP,A,0344006 (ORTHO PHARMACEUTICAL CORP.) 29 November 1989, see the whole document	1,3,14,15,31-37

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° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
28-08-1991	15. 10. 91
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Nuria TORIBIO

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X,P	ANNALS OF THE NEW YORK ACADEMY OF SCIENCE, vol. 616, 1990, (New York, US), D.M. RAUSCH et al.: "Peptides derived from the CDR3-homologous domain of the CD4 molecule are specific inhibitors of HIV-1 and SIV infection, virus-induced cell fusion, and postinfection viral transmission in vitro", pages 125-148, see the abstract; page 140, line 43 - page 143, line 3 ---	1-4,6, 14,15, 31-37
X,P	WO,A,9013562 (GENELABS, INC.) 15 November 1990, see page 16, line 3 - page 26, line 25; claims -----	1-10,14 ,15,31- 37

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 9102832 SA 47631

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 26/09/91
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0344006	29-11-89	AU-A- 3526689	30-11-89
		JP-A- 2131497	21-05-90
WO-A- 9013562	15-11-90	None	